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Enzymatic Solubilization of Heat-denatured Cheese Whey Protein

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ABSTRACT

Resolubilization of heat-denatured cheese whey protein was achieved by partial enzymatic hydrolysis of the protein with food-grade proteases. The efficiency with which porcine, trypsin, papain, and neutral protease from *Bacillus subtilis* solubilized the water-insoluble protein was compared by measuring the percentage of water-soluble nitrogen of the corresponding digests. The tryptic digest was completely soluble at neutral pH and was over 90% water-soluble at pH 6.0. This digest had a pronounced solubility minimum of 65% at pH 4.5. Neutral protease gave a digest with similar pH dependence of the solubility, but the percents of water-soluble nitrogen were all below those of the tryptic digest. Papain gave a digest with maximum solubility at pH 3.0 and approximately 80% solubility at neutral pH. The solubility minimum was again at pH 4.5.

Trypsin proved the most potent protease for solubilizing heat-denatured whey protein. With this enzyme, a water-soluble whey protein preparation was obtained which contained 13.2% nitrogen, 4.53% fat, 2.6% moisture, .23% lactose, and 2.9% ash.

INTRODUCTION

Cheese whey protein is among the most valuable proteins from the nutritional point of view (1). Earlier methods relied on heat precipitation to recover the protein from cheese whey (2). The product obtained by heat precipitation is an essentially water-insoluble gritty powder called lactalbumin. More modern techniques based on ultrafiltration now yield a soluble whey protein product. Despite this achievement, heat precipitation remains of interest because it is simple to perform and yields a high protein product with little contamination by lactose and salts. For a variety of applications such lactalbumin might be the product of choice provided that it could be rendered sufficiently water-soluble.

This report follows an earlier observation on the enzymatic solubilization of heat-denatured whey protein (3) and compares the efficiency of pancreatic trypsin, papain, and a bacterial protease in the solubilization of the protein.

MATERIALS AND METHODS

Preparation of the Whey Protein

Six hundred kilograms of cheese whey from a Gruyere cheese factory were skimmed at pH 6.8 and 38 C in an Alfa Laval centrifuge. After skimming, the whey was acidified to pH 5.0 with 10% HCl (ca. 2500 ml). The whey was heated at 95 C for 20 min with gentle stirring. A fine coagulum appeared. The suspension was cooled and centrifuged in an Alfa Laval centrifuge at 11,000 × *g* and a flow rate of 160 kg/h. The sediment was resuspended in 100 liters of demineralized water and centrifuged again. After three repetitions, 9.7 kg of wet paste were obtained. Lyophilization of the paste was in a Smir Usifroid Lyophilizer and gave 2.60 kg of dry product. The powder contained 14.5% nitrogen, 4.86% fat, 1.5% moisture, .24% lactose, and .09% ash.

Preparation of Water-Soluble Tryptic Digest

Another 600 kg of cheese whey were treated as described above; this time, 13.6 kg of wet paste were obtained. The paste was suspended in 80 liters of demineralized water and allowed to equilibrate at 55 C. After temperature equilibration, the pH was taken to 8.0 with 20% KOH. Three grams of porcine trypsin then

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were added to initiate digestion, during which the pH was kept at 8.0 by automatic addition of KOH.

After 1 h, the suspension already had gone into solution; another 3 g of trypsin were added. After a final readjustment of the pH to 8.0, the reaction was allowed to proceed for another 2 h without further addition of KOH. After 3 h of digestion, the pH had dropped to 7.8; the digest was heated rapidly to 90 C, cooled, and concentrated in a thin film evaporator (Luwa 80) at 30 C. Spray-drying in the IWK 140 (75 C) gave 2.40 kg of dry product. This powder was 13.2% nitrogen, .23% lactose, and 2.90% ash. The α-amino nitrogen content of the product was 11% of its total nitrogen.

Specification of the Enzymes

Porcine trypsin (EC 3.4.4.4.) (4) was from Novo Industries, Copenhagen, Denmark. The preparation was specified as crystalline porcine trypsin 4500 K, 25 Anson units per g. Neutral protease from *Bacillus subtilis* ("Neutrase Novo") was from the same producer and had .5 Anson units per g. Papain (EC 3.4.4.10) (4) was a product of Merck, Darmstadt, Germany. The preparation had 3.5 Anson units per g.

Determination of Water-Soluble Nitrogen

The whey protein digests were dissolved or suspended in distilled water at a concentration of 2% solids. The solution or suspension was divided into seven portions of 30 ml each. The seven samples were adjusted to different pH values ranging from 3.0 to 7.0 with 1 *N* HCl or 1 *N* NaOH. After pH adjustment, the samples were stirred for 1 h at room temperature. No change of pH occurred during this time. Subsequently the samples were centrifuged for 15 min at 3000 rpm in a Heraeus-Christ standard laboratory centrifuge. Fifty microliters of each supernatant were transferred into a tinfoil container and weighed precisely on an electronic Mettler ME 22 microbalance.

The samples then were applied to the automatic sampler of a Carlo Erba 1300 Nitrogen Analyzer (Carlo Erba Instrumentazione, Milano, Italy). The water-soluble nitrogen (SN) was calculated as % SN = 100 × nitrogen content of the supernatant/nitrogen content of the sample.

Automatic Nitrogen Determination

The original Carlo Erba instrument, model 1300, was not adequate for the analyses of liquid samples because of condensation of water vapor in the bimatic value, so a phosphorus pentoxide filter was placed between the reactor and the bimatic valve. This filter absorbed the water of about 30 samples of 50 μl each before it had to be replaced.

The lactose was determined by an enzymatic method (5). The fat was analyzed after acidic hydrolysis of the sample (6). The moisture was analyzed by heating the sample with an infrared lamp under vacuum. The mineral content was determined gravimetrically by ashing at 550 C.

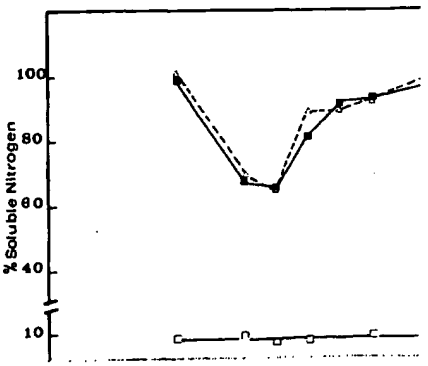
Enzymatic Digestion of Whey Protein

All digestions were at 55 C with suspension of 5% dry matter. The pH for tryptic hydrolysis and papain catalyzed hydrolysis was 8.0 whereas for neutral protease digestions it was 7.0. The enzymatic hydrolysis was in the pH-stat vessel as described earlier (3). The digestions were terminated after 3 h by a brief heating of the digest to 90 C. The pH-stat record indicated near completion of the reaction after 3 h of digestion at 55 C.

RESULTS

Dealing with enzymes of different purity, we had to consider their relative caseinolytic activity to establish the proper enzyme to substrate ratio. The caseinolytic activities (7) were about 1:5:20 for neutral protease:papain: trypsin. We used the enzymes to solubilize the whey protein in the following weight ratio of enzyme to substrate, trypsin 1:200, papain 1:50, and neutral protease 1:16:6; These amounts of enzyme gave a soluble product (at least in the case of trypsin and papain) within a reasonably short time (2 h), and the digestion was not extended over 3 h. However, the indicated enzyme to substrate ratio is not the lowest one which could be used for solubilization. Trypsin solubilized whey protein, even at an enzyme to substrate ratio of 1:1000, provided the digestion was prolonged by about 2 h.

The trypsin and papain digests were more or less transparent solutions, but the neutral protease digest had a milky appearance. We did not observe any off-flavor in any of the three digests, except for a slight bitterness in the tryp-



Enzymatic Solubilization
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TABLE 1. Analytical data on heat-denaturec

Product	Total nitroge
Whey protein	14.5
Tryptic digest[a]	13.8
Tryptic digest[b]	13.2
Papain digest	13.5
Neutral protease digest	13.5

[a]Prepared in the laboratory on small scal
[b]Pilot plant product, prepared from 600

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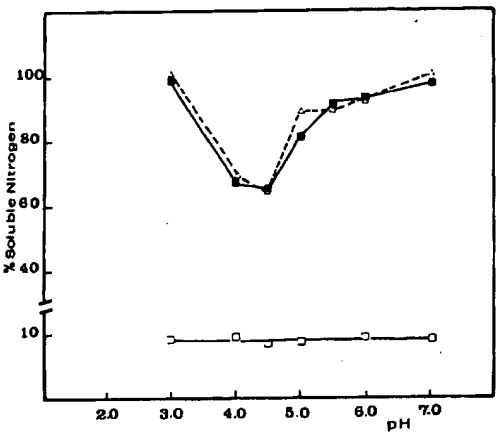

FIG. 1. Water-soluble nitrogen of heat-denatured cheese whey protein (□) and its tryptic digest (■) as a function of pH. Nitrogen was determined with an automatic nitrogen analyzer (■) and with the Kjeldahl method (Δ).

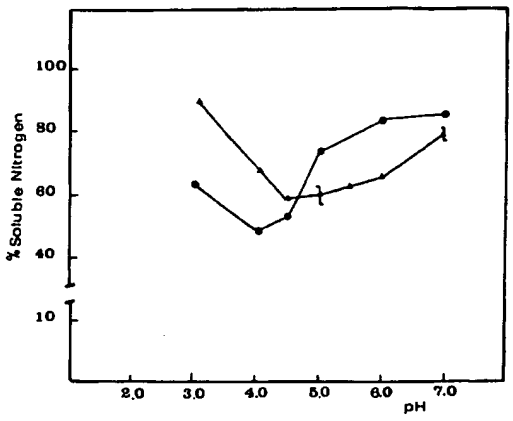

FIG. 2. Water-soluble nitrogen of heat-denatured whey protein digested with neutral protease from *Bacillus subtilis* (●). Water-soluble nitrogen of papain digested, heat-denatured whey protein, as a function of pH (▲). Graphical representation of the means with their confidence intervals at 95% (*f*).

ic and papain digests.

Measurement of the nitrogen solubility over a pH range from 3.0 to 7.0 showed that the tryptic digest was completely soluble at neutral pH and had more than 90% soluble nitrogen at pH 6.0. The solubility curve (Fig. 1) showed a pronounced minimum (65%) at pH 4.5, and at pH 3.0 the solubility was again close to 100%.

The neutral protease digest gave a similar solubility profile (Fig. 2), but the nitrogen solubilities were all lower. In the neutral protease digest, the high solubility at neutral pH had no equivalent on the acidic side as with tryptic digest. Despite a greater extent of hydrolysis, indicated by the higher α-amino nitrogen content of the neutral protease digest (Table 1), this enzyme was apparently the least powerful in solubilization. Papain gave a whey protein digest with maximum nitrogen solubility at pH 3.0 (Fig. 2).

The reproducibility and precision in the determination of the water-soluble nitrogen can be deduced from Table 2. The method is least precise near the solubility minimum of the products. Our measurements seemed highly reproducible and of sufficient precision. In addition, correspondence was close in the values with the nitrogen analyser and the Kjeldahl method (Fig. 1).

TABLE 1. Analytical data on heat-denatured cheese whey protein and its enzymatic digests.

Product	Total nitrogen	α-amino nitrogen	Lactose	Ash
	(%)			
Whey protein	14.5	.7	.24	.09
Tryptic digest[a]	13.8	11.6	.23	2.90
Tryptic digest[b]	13.2	11.0	.21	2.85
Papain digest	13.5	9.0		
Neutral protease digest	13.5	17.8		

[a]Prepared in the laboratory on small scale.

[b]Pilot plant product, prepared from 600 kg of cheese whey.

TABLE 2. Reproducibility of the determination of water-soluble nitrogen for the papain hydrolysate at two different pH values.

	% of water-soluble nitrogen					Σ	x̄	σ	Sm	Lm[a]
pH 5.00	64.3	62.1	56.1	60.0	58.9	360.3	60.1	2.85	1.16	2.98
pH 7.00	78.0	79.3	79.3	76.6	76.6	467.8	78.0	1.21	.49	1.26

[a]Lm = 2.571 × Sm for 95% confidence limits.

DISCUSSION

Efforts to overcome the poor functional properties of heat-denatured whey proteins (lactalbumin) by enzymatic hydrolysis of the protein probably have been made by many workers, but only a few of the results were published. Kuehler and Stine (8) digested whey protein with pronase to obtain an egg-white substitute which could be whipped. In the patent literature the use of trypsin (9) or pancreatin (10) as well as papain (11) is mentioned. The enzyme treatments had different goals: preparation of a soluble hydrolysate (9), preparation of a product which was less sensitive to heat flocculation (10), or preparation of lactalbumin stable in colloidal form (11).

Precise measurements on the solubility of enzymatic lactalbumin digests were not reported. According to our earlier observations (3) and to the present study, heat-denatured whey protein can be solubilized partially by different food-grade proteases. Within certain pH limits, complete solubilization was achieved with trypsin. Solubilization occurred at a rather limited extent of hydrolysis. As in Table 1, the neutral protease digest had the highest α-amino nitrogen content of the three hydrolysates. This indicated that neutral protease produced, on the average, shorter peptides than trypsin or papain. Despite this, the tryptic peptides were the most soluble. It seems possible that trypsin, which attacks hydrophilic sites on the protein, breaks up the substrate molecule in hydrophilic fragments whereas an enzyme which attacks hydrophilic sites is more likely to produce hydrophobic fragments.

Although a slight bitterness was in our digests, the flavor problem was not serious and represented no obstacle to the use of these enzymes. When the heat precipitation of whey protein was combined with subsequent trypsinization of the protein suspension, a soluble product with a high protein content was obtained which seems promising as a food ingredient.

ACKNOWLEDGMENTS

We thank Andre Klein and collaborators for the determinations of fat, lactose, and ash.

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